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13. ABSTRACT (Maximum 200 Words) Prostate cancer (PCa) is the most common invasive malignancy and second leading cause of cancer death in men in the United States. Up until now, hormone ablation therapy is the major way to treat PCa. Such therapy only causes a temporary regression and tumor growth resumes within 6-18 months. Therefore, better androgen blockade is not the answer for treating PCa. Rather, research efforts should focus on the therapeutic agents that will inhibit growth factor signaling pathways thereby inhibit growth. A large number of studies have pointed out that inositol hexaphosphate (IP6) could have beneficial effect on variety of cancers. The specific aims of this proposal are to determine (1) the in vivo effects of IP6 on the growth of PCa (2) the efficacy of IP6 in inhibiting growth factor-induced DNA synthesis of the PCa cells in vitro, and (3) the molecular mechanisms by which IP6 inhibit growth of PCa cells. The information we obtain from these experiments will provide a better understanding of the potential role of IP6 in the prevention of growth of PCa cells. This information will lead to more effective PCa prevention and treatment strategies in human that might prolong the longevity of men with prostate cancer.				
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INTRODUCTION:

Prostate cancer is the most common invasive malignancy and second leading cause of cancer death in men in the United States and many other parts of the world. Up till now, hormone ablation therapy is the major way to treat prostate cancer. Such therapy only causes a temporary regression and tumor growth resumes within 6-18 months. It is now well established that aberrant expressions of mitogenic growth factors and their receptors are responsible for unregulated growth of the prostate cancer. Once autocrine growth factor loops are operative, prostate cancer progresses to an androgen-independent state. It is uniformly fatal because no systemic therapy currently exists that inhibit growth of androgen-independent prostate cancer. Therefore better androgen blockade is not the answer for treating prostate cancer. Rather, research efforts should focus on the therapeutic agents that will inhibit growth factor signaling pathways thereby inhibit growth. While many new classes of cancer chemopreventive agents are being evaluated in clinical trials for other malignancies, little success has been achieved in terms of prostate cancer prevention. During the past several years, a large number of studies have pointed out that inositol hexaphosphate (IP6), the most abundant phosphorylated inositol present in beans, cereal grains, lentils and legumes, could have beneficial effect on variety of cancers. The underlying hypothesis driving our work is that unregulated expression of mitogenic growth factors are responsible for carcinogenesis of the prostate gland and IP6 can prevent such development by inhibiting growth factor-induced signal transduction. Therefore, IP6 could be a potential agent for the prevention and treatment of prostate cancer. The specific aims of this project are to examine (1) the in vivo effects of IP6 on the growth of prostate cancer (2) the efficacy of IP6 in inhibiting growth factor-induced DNA synthesis of prostate cancer cells in vitro, and (3) to determine the molecular mechanisms by which IP6 inhibits growth of prostate cancer cells.

BODY:

In my proposal under the "Statement of Work", I proposed that my first task would be to determine the in vivo effects of inositol hexaphosphate (IP6) on the growth and development of prostate cancer in TRAMP mice. To test the efficacy of IP6 in preventing prostate cancer growth, 36 male TRAMP mice of 4 weeks of age were needed. Although I expected that 36 male TRAMP mice would be generated within 2 months, it took more than 5 months to obtain enough TRAMP male mice to start the treatments. After confirmation by genotyping I started the treatment of TRAMP mice with various doses of IP6 on July 2003. The treatment will be over by the end of March 2004. After the completion of treatments, animals will be sacrificed and tissue will be harvested as proposed in our proposal. So, it will be little bit delayed to finish the task 1 then I proposed. Results from this in vivo study will provide the information whether IP6 can prevent the development of prostate cancer in TRAMP mice. Results will be presented during 2nd Annual report.

Since, the first task took a little bit more time than I anticipated, I initiated two different experiments from Task 2 and Task 3 that we proposed to perform during second and third year. I already standardized a very sensitive and quantitative assay to determine DNA synthesis in TRAMP cell lines by using Chemiluminescence Cell Proliferation (BrdU) ELISA. As seen in Figure 1, IP6 dose dependently decreased BrdU labeling. Similar approach will be used to determine whether IP6 inhibits EGF, TGF- α , IGF-I and bFGF-induced DNA synthesis in TRAMP cancer cells (in Task 2).

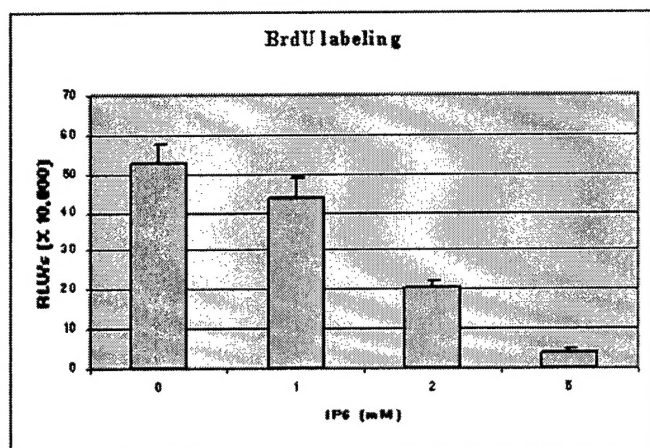


Figure 1. Graph showing the effects of IP6 on BrdU incorporation in TRAMP C2 cells. Five thousand cells were plated in 96 well plate in complete growth media (IMEM containing 10% FBS and antibiotics) with or without various concentrations of IP6. After 22 h of culture BrdU (10 μ M) was added for 2 h and BrdU labeling was determined using Chemiluminescence Cell Proliferation ELISA kit (Roche Diagnostic GmbH, Germany) according to manufacturer's instruction. Data represents the results of triplicates.

To determine the molecular mechanisms by which IP6 inhibits growth of TRAMP prostate cancer cells (under Task 3), we will examine whether IP6 inhibits MAPK and/or PI3K pathways. Since gene transfection and establishment of stable cell lines require longer time, therefore I have already started establishing constitutively active MEK1, MEK2, PI3K and Akt1-transfected stable cell lines. At this point, I have generated two TRAMP cell lines that express higher levels of MEK1 and Akt1 as shown in Figure 2 and other (MEK2 and PI3K) transfected cell lines are in progress. These cell lines will be used in Task 3 to determine the molecular mechanisms of IP6-induced inhibition of prostate cancer cell growth.

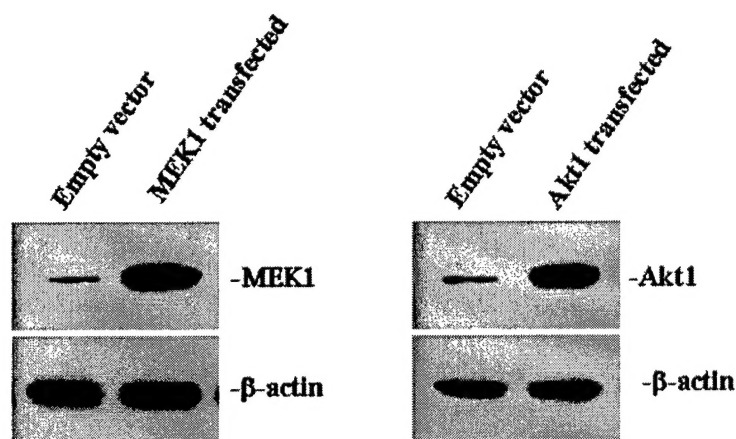


Figure 2. Western blot analyses showing expression of MEK1 and Akt1 in TRAMP C2 cells with empty vector and expression vectors containing constitutively activated MEK1 and Akt1. As we see in these blots, stably transfected cells express higher levels of MEK1 and Akt1. Beta-actin was used as loading control.

KEY RESEARCH ACCOMPLISHMENTS:

- In vivo treatment of IP6 to TRAMP mice is about to complete (Task 1)
- Standardized a highly sensitive BrdU labeling and assay method
- Established stably transfected MEK1 and Akt1 cell lines

REPORTABLE OUTCOMES: I did not anticipate any publication right after the completion of first year. However, during second year several of these experiments will be completed. Therefore, these results will be reported in National Meetings and will be published in peer-reviewed journals during second year.

CONCLUSIONS: So far, all experiments are progressing well as expected. We will make up the delay that have occurred during the initiation of first experiment.

REFERENCES: N/A

APPENDICES: none.